

WHAT IS CLAIMED IS:

1. An isolated protein which is capable of binding to NF- $\kappa$ B regulatory complex and to tumor necrosis factor receptor-associated 2 protein (TRAF2), wherein said protein is not an antibody.

2. The isolated protein of claim 1, which is:

(A) a protein comprising the amino acid sequence of SEQ ID NO:3;

(B) a variant having an amino acid sequence that is at least 85% identical with SEQ ID NO:3; or

(C) a fragment of said protein comprising the amino acid sequence of SEQ ID NO:3 or of said variant (B), wherein said protein, variant and fragment are each capable of binding to the NF- $\kappa$ B regulatory complex and to TRAF2.

3. The isolated protein of claim 2, which is a protein comprising the amino acid sequence of SEQ ID NO:3.

4. The isolated protein of claim 2, which is a fragment of said protein comprising the amino acid sequence of SEQ ID NO:3.

5. An isolated DNA molecule encoding the protein of claim 4.

6. The isolated DNA molecule of claim 5, comprising the nucleotide sequence of SEQ ID NO:1.

7. An expression vector comprising the DNA molecule of claim 5.

8. A host cell transformed with the isolated DNA molecule of claim 5.

9. An isolated DNA molecule encoding the protein of claim 2.

5 10. The isolated DNA molecule of claim 9, wherein said protein comprises the amino acid sequence of SEQ ID NO:3.

11. The isolated DNA molecule of claim 9 which comprises the nucleotide sequence corresponding to nucleotides 497 to 3355 of SEQ ID NO:2.

10 12. An isolated nucleic acid that specifically hybridizes under highly stringent conditions to the complement of said nucleotide sequence of claim 11 corresponding to nucleotides 497 to 3355 of SEQ ID NO:2.

15 13. A pharmaceutical composition comprising the isolated nucleic acid of claim 12 and a pharmaceutically acceptable excipient, diluent, or auxiliary agent.

14. The isolated DNA molecule of claim 9, comprising the nucleotide sequence of SEQ ID NO:2.

20 15. An antisense nucleic acid molecule complementary to the DNA molecule of claim 14, wherein said antisense nucleic acid molecule inhibits the production of a protein capable of binding to the NF-kB regulatory complex and to TRAF2.

25 16. A vector comprising the isolated DNA molecule of claim 9.

17. A host cell transformed with the isolated DNA molecule of claim 9.

18. A method for producing a protein capable of binding to NF- $\kappa$ B regulatory complex and to TRAF2, comprising:

culturing the host cells of claim 17 to produce the protein; and

5 recovering the produced protein.

19. A pharmaceutical composition comprising the isolated DNA molecule of claim 9 and a pharmaceutically acceptable excipient, diluent, or auxiliary agent.

sub C1 20. A pharmaceutical composition comprising the isolated protein of claim 1 and a pharmaceutically acceptable excipient, diluent, or auxiliary agent.

21. A molecule having the binding portion of an antibody capable of binding to the isolated protein of claim 1.

15 22. The molecule of claim 21, which is an antibody.

23. The molecule of claim 22, wherein said antibody is a monoclonal antibody.

sub C1 24. A pharmaceutical composition comprising the molecule of claim 21, and a pharmaceutically acceptable excipient, diluent, or auxiliary agent.

25 25. A method for the modulation or mediation in cells of the activity of NF- $\kappa$ B or any other intracellular signaling activity modulated or mediated by TRAF2 or by other molecules to which a protein, isoform, analog, fragment or derivative thereof according claim 1 binds, comprising treating said cells by introducing thereinto one or more of

09671687 "092800

said protein, isoform, analog, fragment or derivative thereof in a form suitable for intracellular introduction thereof, or introducing into said cells a DNA sequence encoding said one or more protein, isoform, analog, fragment or derivative thereof in the form of a suitable vector carrying said sequence, said vector being capable of effecting the insertion of said sequence into said cells in a way that said sequence is expressed in said cells.

26. A method according to claim 25, wherein said treating of said cells is by transfection of said cells with a recombinant animal virus vector comprising the steps of:

(a) constructing a recombinant animal virus vector carrying a sequence encoding a viral surface protein (ligand) that is capable of binding to a specific cell surface receptor on the surface of said cells to be treated and a second sequence encoding said protein isoform, analog, fragment or derivatives thereof, that when expressed in said cells is capable of modulating/mediating the activity of NF- $\kappa$ B or any other intracellular signaling activity modulated/mediated by TRAF2; and

(b) infecting said cells with said vector of (a).

27. A method for modulating/mediating TRAF2/NF- $\kappa$ B comprising treating cells with a molecule having the binding portion of an antibody according to claim 21, said treatment

being by application of a suitable composition containing said molecule having the binding portion of an antibody to said cells, wherein when the cellular TRAF2/NF- $\kappa$ B complex-binding protein or fragment thereof appears as extracellular surface protein, said composition is formulated for extracellular application, and when said TRAF2/NF- $\kappa$ B complex-binding proteins are intracellular said composition is formulated for intracellular application.

28. A method for modulating/mediating TRAF2/NF- $\kappa$ B comprising treating said cells with the antisense nucleic acid molecule of claim 15, wherein said antisense nucleic acid molecule inhibits the production of the protein capable of binding to the NF- $\kappa$ B regulatory complex and to TRAF2.

29. A method according to claim 28 wherein said antisense oligonucleotide sequence is introduced into said cells by means of an animal virus comprising the same.

30. A method for modulating/mediating TRAF2/NF- $\kappa$ B comprising introducing into a cell a ribozyme sequence capable of interacting with a cellular mRNA sequence encoding a TRAF/NF- $\kappa$ B complex-binding protein according to claim 1, or a vector comprising said ribozyme sequence, whereby expression of said protein is inhibited.

31. A method for isolating and identifying proteins capable of binding directly to TRAF2/NF- $\kappa$ B complex according

09671687, 092800

to claim 1, comprising applying the yeast two-hybrid procedure in which a sequence encoding said TRAF2 is carried by one hybrid vector and sequence from a cDNA or genomic DNA library is carried by the second hybrid vector, the vectors then being  
5 used to transform yeast host cells and the positive transformed cells being isolated, followed by a second two-hybrid test of TRAF2-binding clones for binding to a component of the NF- $\kappa$ B complex, further followed by selection of those clones that bind to TRAF2 and to said component of the  
10 signalosome, and still further, followed by extraction of the said second hybrid vector to obtain a sequence encoding a protein which binds to said TRAF2 and to said component of the signalosome.

32. A method for the prevention or treatment of a  
15 pathological condition associated with NF- $\kappa$ B induction, said method comprising administering to a patient in need an effective amount of a protein or isoform, fragment, analog and derivative thereof or a mixture of any thereof according to claim 1, or a DNA molecule coding therefor, or a molecule  
20 capable of disrupting the interaction of said protein or isoform, fragment, analog and derivative thereof or a mixture of any thereof with TRAF2 or with a component of the signalosome.

33. A method for screening of a ligand capable of binding to a protein according to claim 1 comprising contacting an affinity chromatography matrix to which said protein is attached with a cell extract whereby the ligand is bound to said matrix, and eluting, isolating and analyzing said ligand.

34. A method of screening for a DNA sequence coding for a ligand capable of binding to a protein according to claim 1, comprising applying the yeast two-hybrid procedure in which a sequence encoding said protein is carried by one hybrid vector and sequences from a cDNA or genomic DNA library are carried by the second hybrid vector, transforming yeast host cells with said vectors, isolating the positively transformed cells, and extracting said second hybrid vector to obtain a sequence encoding said ligand.

35. A method for identifying and producing a ligand capable of modulating the cellular activity modulated/mediated by TRAF2 and/or NEMO comprising :

a) screening for a ligand capable of binding to TRAF2;

b) identifying and characterizing a ligand, other than TRAF2 or portions of a receptor of the TNF/NGF receptor family, found by said screening step to be capable of said binding;

c) testing a ligand identified in step (b) for binding to at least a portion of NEMO having the amino acid residues 218 to 416 of NEMO, and selecting a ligand that does bind to said NEMO; and

5 d) producing said ligand in substantially isolated and purified form.

36. A method for identifying and producing a ligand capable of modulating the cellular activity modulated or mediated by a protein according to claim 1, comprising:

10 a) screening for a ligand capable of binding to a polypeptide comprising at least a portion of the amino acid sequence of SEQ ID NO:3;

15 b) identifying and characterizing a ligand, other than TRAF2 found by said screening step to be capable of said binding; and

c) testing a ligand identified in step (b) for binding to at least a portion of NEMO having the amino acid residues 218 to 416 of NEMO, and selecting a ligand that does bind to said NEMO; and

20 d) producing said ligand in substantially isolated and purified form.

37. A method for identifying and producing a molecule capable of directly or indirectly modulating the cellular activity modulated/mediated by NAP, comprising :



a) screening for a molecule capable of modulating activities modulated/mediated by NAP;

b) identifying and characterizing said molecule; and

c) producing said molecule in substantially isolated  
5 and purified form.

add ~~C~~ add D

09671687 092800